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(71) Applicant: **TAKEDA CHEMICAL INDUSTRIES, LTD.**

**1-1, Doshomachi 4-chome
Chuo-ku, Osaka 541(JP)**

(72) Inventor: **Igari, Yasutaka**
**25-503, 4 Motoyaminamimachi 5-chome
Higashinada-ku, Kobe Hyogo 658(JP)**

Inventor: **Yamada, Minoru**
**1-8, Yuyamadai 1-chome
Kawanishi, Hyogo 666-01(JP)**

Inventor: **Ogawa, Yasuaki**
**77-42, Koaza-tanita, Azaohyamazaki,
Ohyamazaki-cho
Otokuni-gun, Kyoto 618(JP)**

(74) Representative: **von Kreisler, Alek,
Dipl.-Chem. et al
Patentanwälte Von Kreisler-Selting-Werner,
Deichmannhaus am Hauptbahnhof
W-5000 Köln 1(DE)**

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(54) **Compositions based on hyaluronic acid.**

(57) A water-soluble composition which comprises (a) a pharmacologically active polypeptide, erythropoietin, secreted by an animal body or its derivative or a chemically synthesized pharmacologically active substance, (b) a water-soluble species of hyaluronic acid or its nontoxic salt and (c) a water-soluble protein injectable into body fluids without showing any substantial pharmacological activity brings about a prolonged action in vivo of a pharmacologically active substance. In addition, the composition can be administered using a small-gauge needle and thereby contributes to relieving pain in patients.

EP 0 522 491 A1

FIELD OF THE INVENTION

The present invention relates to a water-soluble composition which is of value as a sustained-release preparation of a pharmacologically active substance.

BACKGROUND OF THE INVENTION

An object in formulating sustained-release preparations of a pharmacologically active substance, in particular a medicinal peptide, is to maintain the body fluid level of the peptide or the like within a certain effective range over a prolonged period of time. According to the mechanism of manifestation of its pharmacological effect, the peptide or the like may belong to either of the following two classes.

a) The pharmacological effect is not so dependent on the effective body fluid level and, even when the effect is produced to an excessive extent, the living body will not be adversely affected thereby.

b) The pharmacological effect is dependent on the effective body fluid level and abrupt or excessive manifestation of the pharmacological effect is harmful to the living body, so that the dose should be adjusted at regular intervals.

In Japanese Patent Application Laid-open No. 213/1990 is directed to sustained-release preparations containing hyaluronic acid or a nontoxic salt thereof together with a physiologically active peptide. In the examples described therein, a sustained-release calcitonin or elcatonin preparation containing sodium hyaluronate in a concentration of 5% was subcutaneously administered to male rats. Reduction in blood calcium level lasted at least 12 hours. Similarly, in male rats, after administration of a sustained-release human growth hormone preparation containing sodium hyaluronate in a concentration of 5%, the human growth hormone could be detected in the blood over at least 12 hours. In these cases, the duration of activity was evidently prolonged as compared with the control free of sodium hyaluronate. On the other hand, in another comparative example, a tegafur preparation containing sodium hyaluronate in a concentration of 5%, subcutaneously administered to male rats, did not show any prolonged action.

Meanwhile, these inventions make use of retarded diffusion, caused by the viscosity of hyaluronic acid, of a substance in solution at the site of administration. A cationic group-containing substance may undergo ion exchange with the carboxyl-containing hyaluronic acid macromolecule and this ion exchange can further retard the diffusion of the substance. According to Japanese Patent Application Laid-open No. 213/1990, the most preferred concentration of hyaluronic acid itself is 3 to 7%. However, once bubbles are formed due to the high viscosity, elimination thereof becomes a serious problem; centrifugation or application of a reduced pressure will be required for deaeration. At the same time, that high viscosity makes it necessary to use a large-gauge needle and the pain caused in patients is not negligible. Furthermore, even a fairly increased concentration of hyaluronic acid may fail in some instances to produce a prolonged effect, as in the example where tegafur was used.

Japanese Patent Application Laid-open No. 287041/1989 describes an example in which hyaluronic acid was used at a concentration of 1%. The caution in use for Arts Injection (Kaken Pharmaceutical, Japan), which is such a 1% sodium hyaluronate solution for intraarticular injection, recommends the use of a rather large-gauge needle of about 18-20G. As an injectable preparation for subcutaneous administration, such preparation causes much pain in patients.

Japanese Patent Application Laid-open No. 129226/1987 (which corresponds to EP-224 987) describes that the hyaluronic acid concentration in injectable preparations may be within the range of about 0.05% to 4% (by weight) and, in some cases, may be higher depending on the final use of the preparations, without any mention of the applicability of such high concentrations to specific injectable preparations. It can be readily estimated, however, that any prolonged effect of a pharmacologically active substance will not be produced without using hyaluronic acid at a relatively high concentration, as mentioned above. There is indeed the possibility that the effect of a pharmacologically active substance could be prolonged depending on the viscosity of high-concentration hyaluronic acid and that a prolonged effect of a pharmacologically active substance could be similarly produced by utilizing the fact that the hyaluronic acid viscosity can be increased by lowering the pH into the acidic range (e.g. about 2.5). However, the higher the hyaluronic acid viscosity, the more difficult is the administration as an injection. In addition, it is feared that as the pH of the hyaluronic acid solution deviates from the physiological pH range, the stability of the pharmacologically active substance might be affected and/or the tissue at the administration site be damaged.

Japanese Patent Application Laid-open No. 4790/1991 describes that a protein like substance and polysaccharide are added to an aqueous solution of a protease produced by *Bacillus licheniformis* to stabilize the protease. However, there is no teaching or suggestion on the sustained-release of a polypeptide secreted in an animal body or its derivative and a chemically synthesized pharmacologically

active substance.

Although, as mentioned above, attempts have been made to use hyaluronic acid for making up pharmacologically active substances into sustained-release preparations, problems remain to be solved as to the adaptability or compatibility at the time of administration to living bodies.

SUMMARY OF THE INVENTION

The present inventors made intensive investigations in an attempt to solve the above problems and, as a result, found that water-soluble compositions containing a pharmacologically active substance, a water-soluble species of hyaluronic acid and a water-soluble protein injectable into body fluids without showing any substantial pharmacological activity provide, when administered, a prolonged pharmacological effect without loss in the pharmacological activity of the active substance and with suppression of abrupt manifestation of the pharmacological effect at the initial stage following administration. Based on this finding, they made continued investigations and have now completed the present invention.

Thus the invention provides (1) a water-soluble composition which comprises (a) a pharmacologically active polypeptide, except erythropoietin, secreted by an animal body or its derivative or a chemically synthesized pharmacologically active substance, (b) a water-soluble species of hyaluronic acid or its nontoxic salt and (c) a water-soluble protein injectable into body fluids without showing any substantial pharmacological activity; and (2) a method for preparing a water-soluble composition, which comprises mixing (a) a pharmacologically active polypeptide, except erythropoietin, secreted by an animal body or its derivative or a chemically synthesized pharmacologically active substance, (b) a water-soluble species of hyaluronic acid or its non-toxic salt and (c) a water-soluble protein injectable into body fluids without showing any substantial pharmacological activity.

In the present invention, a pharmacologically active polypeptide, except erythropoietin, secreted by an animal body or its derivative or a chemically synthesized pharmacologically active substance is abbreviated as pharmacologically active substance for short.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the serum mutein drug levels, as found in Experimental Example 1.

Figure 2 shows the serum glucose levels, as found in Experimental Example 2.

Figure 3 shows the time course of absolute neutrophil counts in peripheral blood, as found in Experimental Example 3.

Figure 4 shows the serum drug levels, as found in Experimental Example 4.

Figure 5 shows the serum PTH levels, as observed in Experimental Example 6.

DETAILED DESCRIPTION OF THE INVENTION

In the present invention, the pharmacologically active polypeptide secreted by an animal body means a secretory peptide or protein that is produced in an animal body, and secreted internally to blood stream (endocrine) or to surrounding body fluid (paracrine or autocrine) or externally to the outside of the body (exocrine). In addition, the pharmacologically active polypeptide in the present invention means one that is produced by an animal body either as a natural or genetically engineered occurrence.

In the present invention, animal body means an animal comprising a single eukaryotic cell, an animal comprising multiple eukaryotic cells, or a tissue or organ isolated from the animal.

Examples of said animal comprising a single eukaryotic cell include yeast. Examples of said animal comprising multiple eukaryotic cells include a vertebrate animal, an invertebrate animal. And examples of said tissue or organ isolated from the animal include a cancer cell line derived from human or mouse.

Examples of said vertebrate animal include mammals (e.g. human, rodent (e.g. rat, mouse), bovine, horse, sheep), birds (e.g. poultry (e.g. chicken)), fishes (e.g. salmon, tuna), reptile (e.g. snake). Examples of said invertebrate animal include leech, and spider.

In said animal, an animal comprising multiple eukaryotic cells is preferred, and specifically, human, snake, spider and leech are more preferred.

As said polypeptide, one whose partition coefficient measured in octanol over water is below about 0.1 is preferred, and one whose partition coefficient measured in octanol over water is below about 0.05 is more preferred.

As said polypeptide, one having molecular weight of about 200 to 200000 is preferred; and one having molecular weight of about 300 to 90000 is more preferred.

Said polypeptides may be naturally derived ones or ones produced by recombination technique or chemical synthesis.

Examples of the polypeptide include cytokines, peptide hormones, growth factors, the factors that affect cardiovascular system, cell attachment factors, the factors that affect central and peripheral nerve system, the factors that affect body fluid electrolytes and blood organic constituents, the factors that affect bone and skeletal system, the factors that affect gastro-intestinal system, the factors that affect nephro-urinary system, the factors that affect connective tissues and skins, the factors that affect sensory organs, the factors that affect immune system, the factors that affect respiratory system, the factors that affect genital system, and enzymes.

As the polypeptide, cytokines, peptide hormones, growth factors, the factors that affect cardiovascular system, the factors that affect central and peripheral nerve system, the factors that affect body fluid electrolytes and blood organic constituents, the factors that affect bone and skeletal system, the factors that affect gastro-intestinal system, the factors that affect immune system, the factors that affect respiratory system, the factors that affect genital system and enzymes are preferred.

Examples of cytokines include lymphokines, monokines and haematopoietic factors.

Examples of lymphokines include interferons (e.g. alpha, beta, gamma) and interleukins (e.g. IL-2 to IL-11).

Examples of monokines include IL-1, tumor necrotizing factor (e.g. TNF alpha and beta), and leukemic inhibitory factors (LIF).

Examples of haematopoietic factors include, granulocyte colony stimulating factors (G-CSF), granulocyte-macrophage colony stimulating factors (GM-CSF), macrophage colony stimulating factors (M-CSF).

Said haematopoietic factors also include ones that have thrombopoietic growth action, e.g., lymphocyte growth factor formulation (Leukoprol, Morinaga Milk, Japan), thrombopoietin, thrombocyte poiesis stimulatory factor, and megakaryocyte potentiator.

Examples of the factors that affect bone and skeletal system include calcitonin, bone Gla peptide, parathyroid hormone or its active fragment, parathyroid hormone related peptide (PTH-rp) or its active fragment (osteostatin, Endocrinology, 129, 324 (1991)) or histone H4-related osteogenic growth peptide (OGP) (The EMBO Journal, 11, 1867(1992)) or muteins of any of them, or derivatives or analogs of any of them.

Examples of growth factors include nerve growth factor (NGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin like growth factor (IGF), transforming growth factor (TGF), and platelet derived growth factor (PDGF).

Examples of peptide hormone include insulin, growth hormone, leutenizing hormone releasing hormone (LH-RH), adrenocorticotrophic hormone (ACTH), amylin, oxytosin, and leutenizing hormone (LH) or derivatives of any of them.

Examples of the factors that affect cardiovascular system include those that control blood pressure and arteriosclerosis such as endothelin, endothelin inhibitor, vasopressin, renin, angiotensin I, angiotensin II, angiotensin III, angiotensin I inhibitor, angiotensin II receptor antagonist and atrial natriuretic peptide (ANP), and antiarrhythmic peptide.

Examples of the factors that affect central and peripheral nerve system include opioid peptides (e.g., enkephalin, endorphin, kyotorphin), neurotropic factor (NTF), calcitonin gene related peptide, pituitary adenylate cyclase activating polypeptide (PACAP), thyrotropin releasing hormone (TRH) and salts and derivatives thereof (Japanese Patent Application Laid-open 121273/1975 corresponding to U.S. Patent No. 3,959,247 and No. 116465/1977 corresponding to U.S. Patent No. 4,100,152), and neurotensin.

Examples of the factors that affect gastrointestinal system include secretin and gastrin.

Examples of the factors that affect immune system include those that control inflammation and malignant neoplasm and that attacks infective microorganisms.

Examples of the factors that affect body fluid electrolytes and blood organic constituents include those that control blood clotting, plasma cholesterol concentration, and metal ion concentration.

These peptides or factors may further include soluble receptors for the polypeptides.

Examples of cell attachment factors include laminin and inter cellular adhesion molecule (ICAM) 1.

Examples of the polypeptides include natural derived, synthetic or genetically engineered peptides and proteins capable of serving as antigens, e.g., cedar pollen and ragweed pollen. These are administered alone, in a hapten-bound form, or together with an adjuvant in compositions of the present invention, in the form of injections.

Said peptides may further be enzymes of a natural origin or produced by gene recombination techniques, including, but not limited to, superoxidedismutase (SOD), asparaginase and kalikrein, among

other administrable enzymes.

These peptides or factors each may include those chemically modified with synthetic polymer such as polyethyleneglycol, natural polymers such as chondroitin, saccharides, or non-peptide substances. Said non-peptide substances may be either ligands for receptors or antigens for antibodies. In addition, the polypeptides or factors each may include hybrid peptides in which multiple peptides are combined by chemical method or by recombination technique.

Among said polypeptides, interferons, interleukins, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), fibroblast growth factor (FGF), tumor necrotizing factor (TNF), parathyroid hormone (PTH), calcitonin, insulin and leutenizing hormone-releasing hormone (LH-RH) are more preferred.

These peptides or factors each may include muteins differing in carbohydrate chain structure, factors having no carbohydrate chain, analogs or homologs derivatives, thereof, and active fragments derived from these, irrespective of their mechanisms of action, whether antagonistic or agonistic.

Examples of the chemically synthesized pharmacologically active substances include those that affect cardiovascular system, those that affect cell attachment, those that affect central and peripheral nerve system, those that affect body fluid electrolytes and blood organic constituents, those that affect bone and skeletal system, those that affect gastrointestinal system, those that affect nephro-urinary system, those that affect connective tissues and skins, those that affect sensory organs, those that affect immune system, those that affect respiratory system, those that affect infective microorganisms, and those that affect genital system.

As the chemically synthesized pharmacologically active substances, those that affect cardiovascular system, those that affect cell attachment, those that affect central and peripheral nerve system, those that affect body fluid electrolytes and blood organic constituents, those that affect bone and skeletal system, those that affect gastro-intestinal system, those that affect immune system, and those that affect infective microorganisms are preferred.

Examples of the chemically synthesized pharmacologically active substances that affect cardiovascular system include anti-hypertensive agents such as calcium antagonist, angiotensin converting enzyme inhibitor, angiotensin II receptor antagonist and potassium channel opener.

Examples of the chemically synthesized pharmacologically active substances that affect cell attachment include RGD (arginine-glycine-asparaginic acid) antagonist.

Examples of the chemically synthesized pharmacologically active substances that affect central and peripheral nerve system include psychopharmaceuticals and antipsychotic agents such as diazepam, anti nausea-vomitting agent and anti-dementia agents such as benzoquinon derivatives.

Examples of the chemically synthesized pharmacologically active substances that affect body fluid electrolytes and blood organic constituents include those that control blood clotting and plasma cholesterol concentration such as sodium pravastatin.

Examples of the chemically synthesized pharmacologically active substances that affect bone and skeletal system include anti osteoporosis agents such as bis-phosphonates, ipuriflavon, vitamin D3, and antirheumatic agents.

Examples of the chemically synthesized pharmacologically active substances that affect gastrointestinal system includes proton pump inhibitor such as lansoprazol and H₂ receptor antagonist such as cimetidine.

Examples of the chemically synthesized pharmacologically active substances that affect immune system include anti-inflammatory agents such as indomethacin, anti tumor agents such as cis-platinum, adriamycin, anti-allergic agents and nonpeptide antigenic substance.

Examples of the chemically synthesized pharmacologically active substances that affect respiratory system include antiasthmatic agent.

Examples of the chemically synthesized pharmacologically active substances that affect infective microorganisms include antiviral agents and antibiotics such as cephalosporin and penicillin.

Examples of the chemically synthesized pharmacologically active substances that affect genital system include sex steroids such as testosterone.

Examples of the chemically synthesized pharmacologically active substances further include hormone (dexamethasone, predonisolone) and non-peptide antigenic substances.

Said chemically synthesized pharmacologically active substances include inorganic and organic ones.

In addition, these may include prostaglandins, leukotriens, saccharides, polysaccharides, metal chelates, or nucleotides such as antisense RNA or DNA.

Said chemically synthesized nonpeptide antigenic substances are administered alone, in a hapten-bound form, or together with an adjuvant in compositions of the present invention, in the form of injections.

In the present specification, the term "hyaluronic acid" includes hyaluronic acid which is a

mucopolysaccharide composed of N-acetylglucosamine and glucuronic acid, nontoxic salts thereof, hyaluronic acid derivatives and nontoxic salts thereof. The nontoxic salts are, for example, salts with alkali metals, such as sodium and potassium, and salts with alkaline earth metals, such as magnesium and calcium. Among them, the sodium salt is most preferred. Hyaluronic acid and salts thereof preferably have a molecular weight of 1×10^4 to 5×10^6 (as determined by the viscosity method using intrinsic viscosity and the Mark-Houwink equation), more preferably about 1×10^5 to 3×10^6 .

The water-soluble protein injectable into body fluids without showing any substantial pharmacological activity, which is to be incorporated in the composition of the present invention and which is hereinafter sometimes referred to briefly as "water-soluble protein", includes, among others, serum albumin, globulin, collagen, and gelatin. Among them, serum albumin is preferred. The water-soluble protein is preferred intact with no chemical modification.

The term "pharmacological effect" is defined herein as a substantial pharmacological effect caused by water-soluble protein alone at a concentration used in a single dosage unit form.

The water-soluble composition of this invention is adjusted such that when it is dissolved in water, the aqueous solution has a viscosity of not more than about 500 centipoises (cp), preferably within the range of about 50 to 400 cp. Said viscosity depends on the molecular weight and concentration of hyaluronic acid, the concentration of the water-soluble protein, the concentration of the pharmacologically active substance and the concentration of the salt or salts such as sodium chloride (used as the isotonicizing agent to be mentioned later herein), among others, and also depends to some extent on the pH of the water-soluble composition. Therefore, conditions adequate to give the desired water-soluble composition a viscosity within the above range should be selected.

The viscosity referred to herein is measured at a temperature of 25 °C using a viscometer of the type E (TOKIMEC, Japan) with an LD cone.

A preferred range of hyaluronic acid concentration and a preferred range of water-soluble protein concentration in administering the water-soluble composition are mentioned below.

The water-soluble hyaluronic acid concentration at the time of administration should preferably be about 0.01 to 3% (weight to volume), more preferably about 0.05 to 2% (w/v).

When serum albumin, for instance, is used as the water-soluble protein in the composition of the invention, the serum albumin concentration at the time of administration should preferably be about 0.001 to 5% (w/v), more preferably about 0.005 to 2% (w/v). When globulin is used as the water-soluble protein, the globulin concentration at the time of administration should preferably be about 0.001 to 5% (w/v), more preferably about 0.005 to 2% (w/v). When collagen is used as the water-soluble protein, the collagen concentration at the time of administration should preferably be about 0.001 to 1% (w/v), more preferably about 0.005 to 0.2% (w/v). When gelatin is used as the water-soluble protein, the gelatin concentration at the time of administration should preferably be about 0.001 to 1% (w/v), more preferably about 0.005 to 0.5% (w/v).

The pH of a solution prepared from the water-soluble composition of the present invention should be such that said pH will not exert an adverse influence on the activity of the pharmacologically active substance but is within an acceptable range for injections in general and further such that said pH will neither cause a great change in viscosity of the solution nor allow formation of a precipitate or the like. Thus the solution should preferably have a pH of 4 to 8, more preferably a pH of 5 to 8.

As regards the proportion of the pharmacologically active substance in the composition, said substance may be contained therein in an effective amount which may vary depending on the activity of said substance and the therapeutic dose thereof. Generally, the weight ratio between the pharmacologically active substance and hyaluronic acid is preferably from about 0.0001:1 to about 10:1, preferably about 0.0002:1 through about 5:1, more preferably about 0.0002:1 through about 1:1, still more preferably about 0.0002:1 through about 0.1:1.

Although any particular mention cannot be made of the proportion of the water-soluble protein, either, said protein can be added in an amount generally employed in injectable pharmaceutical compositions and a preferred weight ratio between the water-soluble protein and hyaluronic acid is about 0.001:1 to about 100:1, more preferably about 0.01:1 through 10:1, still more preferably about 0.1:1 through about 10:1.

All the three essential components of the composition of the present invention, namely the pharmacologically active substance, hyaluronic acid and water-soluble protein, are preferably made to occur in a single unit dosage form. Thus, for example, the three are made to occur in an ampule or vial by dissolving or suspending them in sterile water or sterile physiological saline. In this case, the method of preparation may comprise admixing a solution of the pharmacologically active substance, a solution of hyaluronic acid and a solution of the water-soluble protein, or adding hyaluronic acid and the water-soluble protein, each in a powder form, to a solution of the pharmacologically active substance, or any other combination of

adequate procedures. The dosage form may also be prepared by adding sterile water or sterile physiological saline to a lyophilizate or vacuum-dried powder in which the pharmacologically active substance, hyaluronic acid and water-soluble protein coexist. This unit dosage form may further contain one or more of conventional additives such as pH adjusting agents (e.g. glycine, hydrochloric acid, sodium hydroxide), local anesthetics (e.g. xylocaine hydrochloride, chlorobutanol), isotonicizing agents (e.g. sodium chloride, mannitol, sorbitol), and adsorption inhibitors (e.g. Tween 80).

This unit dosage form may further contain pharmaceutically acceptable excipients such as polyethylene glycol 400 or dextran.

The goal of admixing the ingredients should be such that the activity of the pharmacologically active substance is maintained and bubble formation is minimized during the process. The ingredients are put into a vessel (for example bottle or drum) either at the same time or in any order. The total volume of the ingredients should be at most three quarters, preferably, three fifths, more preferably half, still more preferably one third of the capacity of the vessel. The vessel is shaken gently and preferably rotated about its longitudinal axis for rotary blending. The number of revolution is selected according to the capacity of the vessel, the total volume of the ingredients, the concentration of hyaluronic acid or its non toxic salt, and so on. The preferred number of revolution ranges from 10 round per minute (rpm) through 1000 rpm, although this is not an exclusive range. The atmosphere in the vessel can be sterile clean air or sterile clean nitrogen gas. The resultant solution can be transferred to small vials or ampules, and can be further subjected to lyophilization.

The water-soluble composition of the present invention preferably takes the form of a parenteral preparation.

As said parenteral preparation, there may be mentioned injectable solutions, solutions for transmucosal administration, nasal solutions, otic solutions, etc.

Said injectable solutions include solutions for intravenous administration, for subcutaneous administration, for intraarticular administration, for intramuscular administration and for intraocular administration, among others. Since the viscosity of these long-acting preparations is adjusted to about 500 cp or less, the preparations can be readily drawn from ampules or vials into syringes using a 25G or 26G needle. Bubbles formed upon the drawing can be readily removed by a short period of mere standing.

The liquid form of the composition of the present invention filled in a soft capsule or the lyophilizate powder of the composition of the present invention filled in a hard capsule or compressed to tablet form may be administered to stomach, large intestine, or rectum.

The composition of the present invention may be in a form dissolved in water or in a lyophilized form with a crystallizing solute such as mannitol.

Addition of sterile water or sterile physiological saline to the lyophilizate gives an aqueous solution.

When the composition is in the form of a lyophilizate, it is required that the viscosity of and the component concentrations in the aqueous solution derived therefrom should be within the respective ranges mentioned hereinbefore.

The liquid form or the lyophilizate powder form of the composition of the present invention dissolved or dispersed in a solution of biodegradable polymer such as poly(lactic-glycolic)acid copolymer, poly(hydroxybutyric acid), poly(hydroxybutyricglycolic) acid copolymer, or the mixture of these can be formulated, for example, to films, microcapsules (microspheres), or nanocapsules (nanospheres) according to the well known methods.

In addition, the composition of the present invention encapsulated in liposomes comprising phospholipids, cholesterol or the derivatives of these according to the well known methods can be further dispersed in physiological saline or the hyaluronic acid solution dissolved in physiological saline.

Further, the composition of the present invention can be supplied in a prefilled syringe for self-administration, since the liquid formulation will not be subject to physical disturbances such as shaking due to its low viscous nature.

The composition of the present invention may be maintained at normal temperature or at normal refrigeration range, preferably about 0°C to a room temperature (e.g. about 25°C), more preferably from about +2°C to +8°C.

The doses, target patients and target diseases of the composition of the present invention correspond to those of the pharmacologically active substance.

The water-soluble composition of the present invention is excellent in producing a prolonged effect. Even a low concentration of hyaluronic acid can produce the effect to a satisfactory extent. As a result, a small-gauge needle can be used, whereby pain in patients can be reduced. The composition has a low viscosity and therefore the possibility of bubble formation is much reduced. Thus the composition can be used with ease in clinical practice.

The following working examples and test examples are further illustrative of the present invention but are by no means limitative of the scope of the invention.

Example 1 Sustained-release preparation containing basic fibroblast growth factor muteine

To 0.36 milliliter of a solution of recombinant human basic FGF muteine CS23 (hereinafter sometimes referred to as rhbFGF muteine CS23) (EP-281,822) (0.96 milligram protein/milliliter) were added 1.26 milliliters of physiological saline for injection and 8.1 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme, U.S.A.), followed by further addition of 6 microliters of Albumin Nichiyaku (Nihon Pharmaceutical, Japan), a 20% solution of human serum albumin. Viscosity: 366 cp.

Example 2

To 0.7 milliliter of physiological saline containing 10 micrograms of nerve cell growth factor (NGF) (Biomedical Technology, U.S.A.) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin, Japan) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 285 cp.

Example 3

To 0.7 milliliter of physiological saline containing 10 micrograms of epithelial growth factor (EGF) (Chemi-Con International, U.S.A.) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 305 cp.

Example 4

To 0.7 milliliter of physiological saline containing 10 micrograms of insulin-like growth factor (IGF) (Chemi-Con International) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 298 cp.

Example 5

To 0.7 milliliter of physiological saline containing 10,000 international units of interferon alpha (Lee Biomolecular Research Labo, U.S.A.) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 312 cp.

Example 6

To 0.7 milliliters of physiological saline containing 10,000 international units of interferon beta (Pesel, Germany) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 320 cp.

Example 7

To 0.7 milliliter of physiological saline containing 10,000 international units of interferon gamma (Genzyme, U.S.A.) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological

saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 311 cp.

5 Example 8

To 0.7 milliliter of physiological saline containing 10 micrograms of interleukin 2 (IL-2) (produced by the method described in Japanese Patent Application Laid-open No. 78799/1986 corresponding to EP-176299 and purified by the method described in Japanese Patent Application Laid-open No. 115528/1985 corresponding to EP-145390; mixture of N-terminal Met-containing species and N-terminal Met-free species) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 320 cp.

Example 9

To 0.7 milliliter of physiological saline containing 1 microgram of transforming growth factor (TGF- β) (Wako Pure Chemical Industries, Japan) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 353 cp.

25 Example 10

To 0.7 milliliter of physiological saline containing 10 micrograms of parathyroid hormone (PTH) (Bachem Fine Chemicals, Switzerland) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 251 cp.

35 Example 11

To 0.7 milliliter of physiological saline containing 5,000 units of granulocyte-macrophage colony stimulating factor (GM-CSF) (ICN Biomedicals, U.S.A.) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 301 cp.

45 Example 12

To 0.7 milliliter of physiological saline containing 5,000 units of macrophage colony stimulating factor (M-CSF) (ICN Biomedicals) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 299 cp.

Example 13

To 0.7 milliliter of physiological saline containing 1 microgram of acid fibroblast growth factor (FGF- α) (Toyobo, Japan) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5

milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 274 cp.

Example 14

To 1 microgram of tumor necrosis factor (TNF- α) (Wako Pure Chemical, Japan) was added 0.7 milliliter of physiological saline for injection (Fuso Yakuhin). To the mixture was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 286 cp.

Example 15

To 0.7 milliliter of physiological saline containing 400 units of Serratia-derived superoxide dismutase (SOD) (Japanese Patent Application Laid-open No. 29285/1982 corresponding to EP-45,222 and No. 16685/1983 corresponding to EP-70,656) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 291 cp.

Example 16

To 0.7 milliliter of physiological saline containing 10 micrograms of vasopressin (Cambridge Research Biochemical, Great Britain) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 220 cp.

Example 17

To 0.7 milliliter of physiological saline containing 10 micrograms of somatostatin (Bachem Fine Chemicals) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 249 cp.

Example 18

To 0.7 milliliter of physiological saline containing 10 micrograms of oxytocin (Bachem Fine Chemicals) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 253 cp.

Example 19

To 0.7 milliliter of physiological saline containing luteinizing hormone releasing hormone (LH-RH) (Bachem Fine Chemicals) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 241 cp.

Example 20

To 0.7 milliliter of physiological saline containing 10 micrograms of growth hormone releasing factor (Bachem Fine Chemicals) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution
 5 was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 265 cp.

Example 21

10 To 0.7 milliliter of physiological saline containing 10 micrograms of growth hormone (UCB Bioproducts, Belgium) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 293 cp.

Example 22

15 To 0.7 milliliter of physiological saline containing 10 micrograms of calcitonin (UCB Bioproducts) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 238 cp.

Example 23

25 To 0.7 milliliter of physiological saline containing 10 micrograms of calcitonin gene-related peptide (UCB Bioproducts) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5
 30 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 229 cp.

Example 24

35 To 0.7 milliliter of physiological saline containing 10 micrograms of brain natriuretic peptide (Peptide Institute, Japan) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5
 40 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 241 cp.

Example 25

45 To 0.7 milliliter of physiological saline containing 10 micrograms of atrial natriuretic peptide (Cambridge Research Biochemicals, U.K.) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 243 cp.

Example 26

50 To 0.7 milliliter of physiological saline containing 10 micrograms of an osteocalcin analog (American Peptide, U.S.A.) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human
 55 serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 265 cp.

Example 27

To 0.7 milliliter of physiological saline containing 10 micrograms of hepatocyte growth factor (Peptide Institute, Japan) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 271 cp.

Example 28

To 0.7 milliliter of physiological saline containing urokinase (Nippon Chemical Research, Japan) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 311 cp.

Example 29

To 0.7 milliliter of physiological saline containing 10 micrograms of tissue plasminogen activator (TPA) (Bioscot, Great Britain) was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 335 cp.

Example 30

To 0.7 milliliter of physiological saline containing 1 microgram of interleukin 3 (IL-3) (R&D Systems, U.S.A.) is added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 259 cp.

Example 31

To 0.7 milliliter of physiological saline containing 1 microgram of interleukin 4 (IL-4) (R&D Systems) is added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 265 cp.

Example 32

To 0.7 milliliter of physiological saline containing 1 microgram of interleukin 5 (IL-5) (Amgen, U.S.A.) is added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin, Japan) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 282 cp.

Example 33

To 0.7 milliliter of physiological saline containing 1 microgram of interleukin 6 (IL-6) (Amgen) is added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 249 cp.

Example 34

To 0.7 milliliter of physiological saline containing 1 microgram of interleukin 7 (IL-7) (Genzyme) is added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 255 cp.

Example 35

To 0.7 milliliter of physiological saline containing 1 microgram of interleukin 8 (IL-8) (Genzyme) is added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%, followed by further addition of 1.4 milliliters of physiological saline for injection (Fuso Yakuhin) to make the whole volume 2.1 milliliters. To this solution was added 10.5 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). Viscosity: 263 cp.

Example 36 Long-acting insulin preparation

Three milligrams of pig insulin (26.8 units/milligram; Diosynth, Netherlands) was dissolved in 1.5 milliliters of 0.1 N hydrochloric acid and then 1.65 milliliters of physiological saline for injection was added, followed by addition of 15.75 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). To the thus-obtained solution was added 4 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%. Viscosity: 384 cp.

Example 37 Long lasting preparation containing granulocyte colony stimulating factor (G-CSF)

To 0.0466 milliliter of a human granulocyte colony stimulating factor preparation (Nupogen; Amgen, U.S.A.) containing, per milliliter, 300 micrograms of recombinant human G-CSF, 50 milligrams of mannitol and 0.004% of Tween 80 was added 4.154 milliliters of physiological saline for injection, followed by further addition of 21 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). To the thus-obtained solution was added 8 microliters of Albumin Nichiyaku (Nihon Pharmaceutical) containing human serum albumin at a concentration of 20%. Viscosity: 264 cp.

Example 38 Long-acting insulin preparation

Three milligrams of pig insulin (26.8 units/milligram; Diosynth) was dissolved in 1.5 milliliters of 0.1 N hydrochloric acid and then 1.65 milliliters of physiological saline for injection was added, followed by further addition of 15.75 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme). To the solution obtained was added 0.5 milligram of human immunoglobulin G (Cappel). Viscosity: 328 cp.

Example 39 Long-acting granulocyte colony stimulating factor (G-CSF) preparation

To 0.0233 milliliters of a human granulocyte colony stimulating factor preparation (Nupogen; Amgen, U.S.A.) containing, per milliliter, 300 micrograms of a recombinant human G-CSF, 50 milligrams of mannitol and 0.004% of Tween 80 was added 1.377 milliliters of physiological saline for injection, followed by further addition of 8 microliters of Albumin Nichiyaku (Nippon Seiyaku) containing 20% of human serum albumin. To the resultant mixture was added 0.7 milliliter of a 1.5% (w/v) solution of sodium hyaluronate (average molecular weight: 1,470,000 daltons; Genzyme, U.S.A.) in physiological saline for injection (Fuso Pharmaceutical). A glass vial (capacity: ca. 5 milliliters) containing these solutions was tightly stoppered and rotated, for rotary blending, in a 200-milliliter eggplant-shaped flask equipped with a three-one motor (Heydon, Japan) about its longitudinal axis (20-100 revolutions per minute (rpm)) for about one hour. The above procedure gave a long-acting dosing solution substantially free of bubbles.

Example 40

To 0.023 milliliter of a human granulocyte colony stimulating factor preparation (Nupogen; Amgen) containing, per milliliter, 300 micrograms of a recombinant human G-CSF, 50 milligrams of mannitol and 0.004% of Tween 80 was added 1.377 milliliters of physiological saline for injection, followed by further

addition of 4 microliters of Albumin Nichiyaku (Nippon Seiyaku) containing 20% of human serum albumin. To the resultant mixture was added 0.7 milliliter of a 1.5% (w/v) solution of sodium hyaluronate (average molecular weight: ca. 500,000 daltons) in physiological saline for injection (Fuso Pharmaceutical). A glass vial (capacity: ca. 5 milliliters) containing these solutions was hermetically stoppered and subjected to rotary blending in a 200-milliliter eggplant-shaped flask equipped with a three-one motor (Heydon) by rotation (20-100 rpm) about its longitudinal axis for about one hour. The above procedure gave a long-acting dosing solution substantially free of bubbles.

Example 41

To 0.023 milliliter of a human granulocyte colony stimulating factor preparation (Nupogen; Amgen) containing, per milliliter, 300 micrograms of a recombinant human G-CSF, 50 milligrams of mannitol and 0.004% of Tween 80 was added 1.039 milliliters of physiological saline for injection, followed by further addition of 4 microliters of Albumin Nichiyaku (Nippon Seiyaku) containing 20% of human serum albumin. To the resultant mixture was added 1.039 milliliters of a 1% solution of sodium hyaluronate (average molecular weight: ca. 800,000-900,000 daltons; Artz Injection; Seikagaku Corporation, Japan). A glass vial (capacity: ca. 5 milliliters) containing these solutions was tightly stoppered and subjected to rotary blending in a 200-milliliter eggplant-shaped flask equipped with a three-one motor (Heydon) by rotation (20-100 rpm) about its longitudinal axis for about one hour. The above procedure gave a long-acting dosing solution substantially free of bubbles.

Example 42

To 0.023 milliliter of a human granulocyte colony stimulating factor preparation (Nupogen; Amgen) containing, per milliliter, 300 micrograms of a recombinant human G-CSF, 50 milligrams of mannitol and 0.004% Tween 80 was added 1.377 milliliters of physiological saline for injection, followed by further addition of 4 microliters of Albumin Nichiyaku (Nippon Seiyaku) containing 20% of human serum albumin. To the resultant mixture was added 0.7 milliliter of a 1.2% (w/v) solution of sodium hyaluronate (average molecular weight: 1,800,000 daltons; Genzyme) in physiological saline for injection (Fuso Pharmaceutical). A glass vial (capacity: ca. 5 milliliters) containing these solutions was tightly stoppered and subjected to rotary blending in a 200-milliliter eggplant-shaped flask equipped with a three-one motor (Heydon) by rotation (20-100 rpm) about its longitudinal axis for about one hour. The above procedure gave a long-acting dosing solution substantially free of bubbles.

Example 43

To 0.023 milliliter of a human granulocyte colony stimulating factor preparation (Nupogen; Amgen) containing, per milliliter, 300 micrograms of a recombinant human G-CSF, 50 milligrams of mannitol and 0.004% of Tween 80 was added 1.377 milliliters of physiological saline for injection, followed by further addition of 4 microliters of Albumin Nichiyaku (Nippon Seiyaku) containing 20% of human serum albumin. To the resultant mixture was added 0.7 milliliter of a 0.9% (w/v) solution of sodium hyaluronate (average molecular weight: ca. 2,300,000 daltons; Genzyme) in physiological saline for injection (Fuso Pharmaceutical). A glass vial (capacity: ca. 5 milliliters) containing these solutions was hermetically stoppered and subjected to rotary blending in a 200-milliliter eggplant-shaped flask equipped with a three-one motor (Heydon) by rotation (20-100 rpm) about its longitudinal axis for about one hour. The above procedure gave a long-acting dosing solution substantially free of bubbles.

Example 44

In 1 milliliter of physiological saline for injection was dissolved 300 micrograms of an active human parathyroid hormone (PTH) fragment (Bachem Fine Chemicals, Switzerland) covering from the amino terminal to the 34th amino acid of PTH, followed by addition of 4 microliters of Albumin Nichiyaku (Nippon Seiyaku) containing 20% of human serum albumin. To the resultant mixture was added 1.5 milliliters of a 1.2% (w/v) solution of sodium hyaluronate (average molecular weight: ca. 1,800,000 daltons; Genzyme) in physiological saline (Fuso Pharmaceutical). A glass vial (capacity: ca. 10 milliliters) containing these solutions was subjected to rotary blending in a 200 milliliter eggplant-shaped flask equipped with a three-one motor (Heydon) by rotation (20-100 rpm) about its longitudinal axis for about one hour. The above procedure gave a long-acting dosing solution substantially free of bubbles.

Example 45

In 1 milliliter of physiological saline for injection was dissolved 300 micrograms of human parathyroid hormone (PTH) (Bachem Fine Chemicals), followed by addition of 4 microliters of Albumin Nichiyaku (Nippon Seiyaku) containing 20% of human serum albumin. To the resultant mixture was added 1.5 milliliters of a 1.2% (w/v) solution of sodium hyaluronate (average molecular weight: ca. 1,800,000 daltons; Genzyme) in physiological saline for injection (Fuso Pharmaceutical). A glass vial (capacity: ca. 10 milliliters) containing these solutions was tightly closed and subjected to rotary blending in a 200-milliliter eggplant-shaped flask equipped with a three-one motor (Heydon) by rotation (20-100 rpm) about its longitudinal axis for about one hour. The above procedure gave a long-acting dosing solution substantially free of bubbles.

Example 46

To a solution containing 100 ATU of hirudin (Peninsula Laboratories Inc., U.S.A.) is added physiological saline for injection to make the whole volume 1.4 milliliters. Then, 4 microliters of Albumin Nichiyaku (Nippon Seiyaku) containing 20% of human serum albumin is added. To the resultant mixture is added 0.7 milliliter of a 1.2% (w/v) solution of sodium hyaluronate (average molecular weight: ca. 1,800,000 daltons; Genzyme) in physiological saline (Fuso Pharmaceutical). A glass vial (capacity: ca. 5 milliliters) containing these solutions is tightly stoppered and subjected to rotary blending in a 200-milliliter eggplant-shaped flask equipped with a three-one motor (Heydon) by rotation (20-100 rpm) about its longitudinal axis for about one hour. The above procedure gives a long-acting dosing solution substantially free of bubbles.

Example 47

In 1.4 milliliters of physiological saline was dissolved 10 milligrams of thyrotropic hormone releasing hormone (TRH; Bachem Fine Chemicals, Switzerland), followed by addition of 4 microliters of Albumin Nichiyaku (Nippon Seiyaku) containing 20% of human serum albumin. To the resultant mixture was added 0.7 milliliter of a 1.2% (w/v) solution of sodium hyaluronate (average molecular weight: ca. 1,800,000 daltons; Genzyme) in physiological saline for injection (Fuso Pharmaceutical). A glass vial (capacity: ca. 5 milliliters) containing these solutions was hermetically stoppered and subjected to rotary blending in a 200-milliliter eggplant-shaped flask equipped with a three-one motor (Heydon) by rotation (20-100 rpm) about its longitudinal axis for about one hour. The above procedure gave a long-acting dosing solution substantially free of bubbles.

Example 48

In 1.4 milliliters of physiological saline was dissolved 10 milligrams of cefotiam dihydrochloride (Takeda Chemical Industries, Japan), followed by addition of 4 microliters of Albumin Nichiyaku (Nippon Seiyaku) containing 20% of human serum albumin. To the resultant mixture was added 0.7 milliliter of a 1.2% (w/v) solution of sodium hyaluronate (average molecular weight: ca. 1,800,000 daltons; Genzyme) in physiological saline for injection (Fuso Pharmaceutical). A glass vial (capacity: ca. 5 milliliters) containing these solutions was tightly stoppered and subjected to rotary blending in a 200-milliliter eggplant-shaped flask equipped with a three-one motor (Heydon) by rotation (20-100 rpm) about its longitudinal axis for about one hour. The above procedure gave a long-acting dosing solution substantially free of bubbles.

Example 49

To each of two vials of Canferon-A300 (Takeda Chemical, Japan) containing 3 million international units (IU) of interferon alfa-2a and 5 mg of human serum albumin was added 1 ml of distilled water to provide an interferon alfa injection. 1.4 ml of the interferon alfa injection prepared as above (containing 4.2 million IU of interferon alfa-2a) and 0.7 ml of 1.5% (w/v) physiological saline solution of sodium hyaluronate (average molecular weight, 1,470,000 daltons) were put into a glass vial (capacity: ca. 5 ml). The glass vial containing these solutions was hermetically closed and subjected to rotary blending in a 200 ml egg-plant-type flask equipped with a three-one motor (Heydon, Japan) by rotation about its longitudinal axis (20-100 rpm) for about 1 hour. The procedure gave a long-acting dosing solution substantially free of bubbles.

Example 50

To 0.4 milliliter of a solution of recombinant human basic FGF mutein CS23 (EP-281,822)(0.96 milligram protein/milliliter) were added 2.4 milliliters of physiological saline for injection and 15 microliters of Albumin Nichiyaku (Nihon Pharmaceutical, Japan), a 20% solution of human serum albumin, followed by addition of 1.4 milliliters of 1.5% (w/v) physiological saline solution of sodium hyaluronate (average molecular weight, 1,470,000 daltons). A glass vial (capacity: ca. 12 ml) was hermetically closed and subjected to rotary blending in a 200 ml egg-plant type flask equipped with a three-one motor (Heydon, Japan) by rotation about its longitudinal axis (20-100 rpm) for about 1 hour. The procedure gave a long acting dosing solution substantially free of bubbles.

Example 51

In 2 ml of physiological saline for injection (Fuso Pharmaceutical, Japan) was added 300 micrograms of parathyroid hormone (PTH) (Bachem Fine Chemicals). A glass vial (capacity: ca. 5 ml) filled with the above injection was added 4 microliters of Nichiyaku Albumin (Nihon Seiyaku) containing 20% (v/v) of human serum albumin and 1 ml of a 1.5% (w/v) solution of sodium hyaluronate (average molecular weight 1,470,000; Genzyme) in physiological saline. The glass vial containing these solutions was hermetically closed and subjected to rotary blending by rotation about its longitudinal axis (20-100 rpm) in a three-one motor (Heydon; Japan) for about one hour. The procedure gave a long-acting dosing solution substantially free of bubbles.

Experimental Example 1

The following two injections were further prepared and subjected to testing.

Comparative Preparation 1

To 0.35 milliliter of a solution of recombinant human basic FGF mutein CS23 (EP-281,822) (0.96 milligram protein/milliliter) was added 1.225 milliliters of physiological saline for injection.

Comparative Preparation 2

To 0.36 milliliter of a solution of recombinant human basic FGF mutein CS23 (EP-281,822) (0.96 milligram protein/milliliter) were added 1.26 milliliters of physiological saline for injection and 8.1 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme).

The injection of Example 1 was subcutaneously administered to 8-week-old male SD rats on their back at a dose of 160 micrograms/kilogram. As controls, Comparative Preparation 1 and Comparative Preparation 2 were administered in the same manner. Before administration and at regular intervals after administration, about 0.4-milliliter portions of blood were collected. The serum was separated from each blood sample and assayed for serum rhbFGF mutein CS23 level by ELISA. The results obtained are shown in Fig. 1. The figure graphically shows the courses of change in blood mutein level after administration of Comparative Preparation 1(o), Comparative Preparation 2(□), and the rhbFGF mutein CS23 injection of Example 1 (●), respectively. Rats were used in groups of 5. In each graph, each dot indicates a mean value and each bar a standard error (S.E.).

It was found that while rhbFGF mutein CS23 rapidly disappeared from the blood in the group given Comparative Preparation 1 (simple rhbFGF mutein CS23 solution), the blood rhbFGF mutein CS23 level was maintained high in the group given Comparative Preparation 2 containing hyaluronic acid owing to the effect of hyaluronic acid. It was found, however, that the blood mutein level was maintained still higher at hour 1 and hour 4 after administration in the group given the injection of Example 1 prepared by adding hyaluronic acid and serum albumin to rhbFGF mutein CS23 as compared with the group given Comparative Preparation 2 prepared by adding hyaluronic acid alone.

Experimental Example 2

The following two injections were further prepared and subjected to testing.

Comparative Preparation 3

Three milligrams of pig insulin (26.8 units/milligram; Diosynth) was dissolved in 1.5 milliliters of 0.1 N

hydrochloric acid and then 1.65 milliliters of physiological saline for injection was added, followed by addition of 15.75 milligrams of sodium hyaluronate (average molecular weight 1,470,000 daltons; Genzyme).

Comparative Preparation 4

Physiological saline for injection 2 milliliters

The insulin injection of Example 36 and Comparative Preparation 3 were respectively administered subcutaneously to 8-week-old male SD rats on their back at a dose of 200 micrograms/rat. In a control group, Comparative Preparation 4, namely physiological saline for injection, was administered at the same voluminal dose as used in administering the preparation of Example 36 and Comparative Preparation 3. Before administration and at regular intervals after administration, about 0.5-milliliter portions of blood were collected and the serum samples derived therefrom were assayed for serum glucose level using a glucose C test kit (Wako Pure Chemical, Japan).

The results obtained are shown in Fig. 2. The figure shows the time courses of the serum glucose level as respectively revealed in the group given Comparative Preparation 4 (physiological saline) (o), the group given Comparative Preparation 3 (solution of pig insulin plus hyaluronic acid) (o colored black) and in the group given the solution of Example 36 containing pig insulin plus a combination of hyaluronic acid and serum albumin (Δ). Rats were used in groups of 5 and, in Fig. 2, each dot indicates a mean value. In the group given Comparative Preparation 4 (physiological saline), the serum glucose level remained almost unchanged. In the groups given Comparative Preparation 3 and the preparation Example 36, respectively, the serum glucose level was suppressed to substantially the same extent until minute 240 postadministration. At minute 360 postadministration and thereafter, the serum glucose level reduction lasted for a longer period in the group given the preparation of Example 36. At minute 540 postadministration, the serum glucose level had substantially returned to a normal value range in the Comparative Preparation 3 group while, in the Example 36 group, the level was still about two thirds of that in the physiological saline (Comparative Preparation 4) group. This indicates that while the combination of insulin and hyaluronic acid is said to prolong the effect of the peptide, further addition of serum albumin to this system can cause the pharmacological effect to last still longer at the same dose of insulin and in the same amount of hyaluronic acid.

Experimental Example 3

The following injections were further prepared and tested.

Comparative Preparation 5

To 0.0233 milliliter of a human granulocyte colony stimulating factor preparation (Nupogen; Amgen) containing, per milliliter, 300 micrograms of a recombinant human G-CSF, 50 milligrams of mannitol and 0.004% of Tween 80 was added 1.377 milliliters of physiological saline for injection. To the resultant mixture was added 0.7 milliliter of a 1.5% (w/v) solution of sodium hyaluronate (average molecular weight: 1,470,000 daltons; Genzyme) in physiological saline (Fuso Pharmaceutical). A glass vial (capacity: ca. 5 milliliters) containing these solutions was hermetically stoppered and subjected to rotary blending in a 200-milliliter eggplant-shaped flask equipped with a three-one motor (Heydon) by rotation (20-100 rpm) about its longitudinal axis for about one hour. The above procedure gave a long-acting dosing solution substantially free of bubbles.

Comparative Preparation 6

To 0.0233 milliliter of a human granulocyte colony stimulating factor preparation (Nupogen; Amgen) containing, per milliliter, 300 micrograms of a recombinant human G-CSF, 50 milligrams of mannitol and 0.004% of Tween 80 was added 2.077 milliliters of physiological saline for injection.

Comparative Preparation 7

To 1.4 milliliters of physiological saline for injection was added 0.7 milliliter of a 1.5% (w/v) solution of sodium hyaluronate (average molecular weight: 1,470,000 daltons; Genzyme) in physiological saline for injection (Fuso Pharmaceutical). A glass vial (capacity: ca. 5 milliliters) containing these solutions was

hermetically closed and subjected to rotary blending in a 200-milliliter eggplant-shaped flask equipped with a three-one motor (Heydon) by rotation (20-100 rpm) about its longitudinal axis for about one hour. The above procedure gave a long-acting dosing solution substantially free of bubbles.

Eight-week-old male SD rats were subcutaneously dosed by injection at their back, with the human G-CSF-containing preparation of Example 39, Comparative Preparation 5 or Comparative Preparation 6 in a volume of 0.3 milliliter. In a control group, the solution of sodium hyaluronate in physiological saline for injection (Comparative Preparation 7) was administered in a volume of 0.3 milliliter. Before administration and serially after administration, about 0.1 milliliter of blood was withdrawn (EDTA*2Na used as anticoagulant). Peripheral leukocytes, erythrocytes and platelets were counted using a CC-180A microcell counter (Toa Iyo Denshi, Japan). Peripheral neutrophil, lymphocyte, monocyte and eosinophil counts were estimated by multiplying the respective cell occurrence frequencies, as found by typing, under a microscope, of 200 leukocytes for each Giemsa-stained smear preparation, by the leukocyte count.

The results thus obtained are shown in Fig. 3. In the control group (o) given the aqueous hyaluronic acid solution (Comparative Preparation 7), the peripheral blood neutrophil count changed little. In the positive control group (o colored black) given the human G-CSF solution (Comparative Preparation 6), the peripheral blood 16 hours after administration showed a nearly doubled neutrophil count as compared with the control group. In the group (Δ) given Comparative Preparation 5, which contained hyaluronic acid additionally, the peripheral blood showed a further increase in neutrophil count. In the group (\square) given the preparation of Example 39, which contained human G-CSF in combination with hyaluronic acid plus human serum albumin, the peripheral blood showed a still further increase in neutrophil count as compared with the group given Comparative Preparation 5. At 24 hours after administration, the peripheral blood neutrophil counts in the groups given Comparative Preparation 5 and Comparative Preparation 6, respectively, were already at substantially the same level as in the control group whereas, in the group given the preparation of Example 39, the neutrophil count was still approximately twice the level in the control group. These results indicate that the addition of human serum albumin can further potentiate the pharmacological effect prolonging action of hyaluronic acid. In the figure, each point indicates a mean of 5 rats.

Experimental example 4

The following injection was further prepared and tested.

Comparative Preparation 8

To each of two vials of Canferon-A300 (Takeda Chemical, Japan) containing 3 million international units (IU) of interferon alfa-2a and 5 mg of human serum albumin was added 1 ml of distilled water to provide an interferon alfa injection. 1.4 ml of the interferon alfa injection prepared as above (containing 4.2 million IU of interferon alfa-2a) and 0.7 ml of physiological saline for injection were put into a glass vial (capacity: ca. 5 ml) and mixed gently.

Eight-week-old male SD rats were subcutaneously dosed by injection at their back, with the interferon alfa-2a-containing preparation of Example 49 or Comparative Preparation 8 in volume of 0.3 milliliter. Before administration and serially after administration, about 0.4 ml portions of blood were collected. The serum was separated from each blood sample and assayed for serum interferon alfa 2-a level by ELISA.

The results are plotted in Fig. 4. Interferon alfa 2-a was rapidly absorbed from the injection site in the control group treated with Comparative Preparation 8 and the serum interferon alfa 2-a concentration fell with time (o). On the other hand, in the group treated with the preparation of Example 49 containing hyaluronic acid, human serum albumin, and interferon alfa 2-a, the serum interferon alfa 2-a concentrations were higher compared with those of the control group from 1 hour till 8 hours after administration (\bullet). These results manifest the positive effect of the combination of hyaluronic acid, human serum albumin, and interferon alfa 2-a in sustaining the serum drug concentrations. Each point represents the mean of five rats.

Experimental Example 5

The following composition was further prepared and tested.

Comparative Preparation 9

To 0.4 milliliter of a solution of recombinant human basic FGF mutein CS23(EP-281,822) (0.96 milligram protein/milliliter) was added 3.6 milliliters of physiological saline for injection.

Eight-week-old male SD rats fasted for 24 hours were lightly anesthetized by intraperitoneal injection of sodium pentobarbital (Somnopenyl, Pitman Moore, U.S.A.) and were orally administered 1 milliliter of either the preparation of Example 50 or the preparation of Comparative Preparation 9 to stomach. At 30 minutes post administration, incision was made at abdomen and the content of the stomach was collected. Portion of the collected content was immediately centrifuged (Microfuge B, Beckman, U.S.A.), and a 50 microliters aliquot of the supernatant was mixed with a 100 microliters aliquot of a protease inhibitor (Aprotinin, Sigma, U.S.A.) and stored at -40 °C for further determination of CS23 concentration by enzyme immuno assay.

	CS23 Concentration (pg/ml)
Comparative Preparation 9	1768
Preparation of Example 50	8448

The results manifest the positive effect of the combination of hyaluronic acid, human serum albumin, and recombinant human basic FGF mutein CS23, in persisting in succus gastricus inside stomach.

Experimental Example 6

The following composition was further prepared and tested.

Comparative Preparation 10

In 2 ml of physiological saline for injection (Fuso Pharmaceutical) was added 300 micrograms of parathyroid hormone (Bachem Fine Chemicals).

The composition of Example 51 (●) and Comparative Preparation 10 (○) were respectively administered subcutaneously at the back of 8-week-old male SD rats in a volume of 0.2 ml. Before administration and serially after administration, about 0.4 ml of blood was withdrawn and serum sample was separated for the determination of PTH by radio immunoassay. As shown in Fig. 5, at 2 hours and 3 hours post administration the serum PTH level was significantly higher in the group of given Example 51 than in the group given Comparative Preparation 10.

The results manifest the positive effect of the combination of hyaluronic acid, human serum albumin, and PTH in prolonging the serum level of PTH.

Claims

1. A water-soluble composition which comprises (a) a pharmacologically active polypeptide, except erythropoietin, secreted by an animal body or its derivative or a chemically synthesized pharmacologically active substance, (b) a water-soluble species of hyaluronic acid or its non-toxic salt and (c) a water-soluble protein injectable into body fluids without showing any substantial pharmacological activity.
2. A water-soluble composition as claimed in Claim 1, wherein the pharmacologically active polypeptide is cytokines, peptide hormones, growth factors, the factors that affect cardiovascular system, the factors that affect central and peripheral nerve system, the factors that affect body fluid electrolytes and blood organic constituents, the factors that affect bone and skeletal system, the factors that affect gastrointestinal system, the factors that affect immune system, the factors that affect respiratory system, the factors that affect genital system or enzymes, or its mutagen, derivative, analog, homolog, or active fragment.
3. A water-soluble composition as claimed in Claim 11, wherein the pharmacologically active polypeptide is interferons, interleukins, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), fibroblast growth factor (FGF), tumor necrotizing factor (TNF), parathyroid hormone (PTH), calcitonin, insulin or leutenizing hormone-releasing hormone (LH-RH).
4. A water-soluble composition as claimed in Claim 1, wherein the chemically synthesized pharmacologically active substance is those that affect cardiovascular system, those that affect cell attachment, those that affect central and peripheral nerve system, those that affect body fluid electrolytes and blood organic constituents, those that affect bone and skeletal system, those that affect gastro-intestinal

system, those that affect immune system or those that affect infective microorganism.

5. A water-soluble composition as claimed in claim 1, wherein the water-soluble protein is human serum albumin.
6. A water-soluble composition as claimed in Claim 1, which shows a viscosity of not more than 500 cp in an aqueous solution form.
7. A water-soluble composition as claimed in, Claim 1, which is in the form of preparation for parenteral administration.
8. A water-soluble composition as claimed in Claim 1 which is in the form of an injectable preparation.
9. A water-soluble composition as claimed in Claim 1, wherein the weight ratio of the pharmacologically active substance to hyaluronic acid or its nontoxic salt is in the range of about 0.0001 : 1 to about 10 : 1, and the weight ratio of water-soluble protein to hyaluronic acid or its nontoxic salt is in the range of about 0.001 : 1 to about 100 : 1.

Claims for the following Contracting States : GR, ES

1. A method for preparing a water-soluble composition, which comprises mixing (a) a pharmacologically active polypeptide, except erythropoietin, secreted by an animal body or its derivative or a chemically synthesized pharmacologically active substance, (b) a water-soluble species of hyaluronic acid or its non-toxi salt and (c) a water-soluble protein injectable into body fluids without showing any substantial pharmacological activity.
2. A method as claimed in Claim 1, wherein the pharmacologically active polypeptide is cytokines, peptide hormones, growth factors, the factors that affect cardiovascular system, the factors that affect central and peripheral nerve system, the factors that affect body fluid electrolytes and blood organic constituents, the factors that affect bone-and skeletal system, the factors that affect gastro-intestinal system, the factors that affect immune system, the factors that affect respiratory system, the factors that affect genital system or enzymes, or its mutain, derivative, analog, homolog, or active fragment.
3. A method as claimed in Claim 1, wherein the pharmacologically active polypeptide is interferons, interleukins, granulocyte colony stimulating factor (G-CSF), granylocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), fibroblast growth factor (FGF), tumor necrotizing factor (TNF), parathyroid hormone (PTH), calcitonin, insulin or leutenizing hormone-releasing hormone (LH-RH).
4. A method as claimed in Claim 1, wherein the chemically synthesized pharmacologically active substance is those that affect cardiovascular system, those that affect cell attachment, those that affect central and peripheral nerve system, those that affect body fluid electrolytes and blood organic constituents, those that affect bone and skeletal system, those that affect gastro-intestinal system, those that affect immune system or those that affect infective microorganism.
5. A method as claimed in claim 1, wherein the water-soluble protein is human serum albumin.
6. A method as claimed in Claim 1, which shows a viscosity of not more than 500 cp in an aqueous solution form.
7. A method as claimed in Claim 1, which is in the form of preparation for parenteral administration.
8. A method as claimed in Claim 1, which is in the form of an injectable preparation.
9. A method as claimed in Claim 1, wherein the weight ratio of the pharmacologically active substance to hyaluronic acid or its nontoxic salt is in the range of about 0.0001 : 1 to about 10 : 1, and the weight ratio of water-soluble protein to hyaluronic acid or its nontoxic salt is in the range of about 0.001 : 1 to about 100 : 1.

Fig. 1

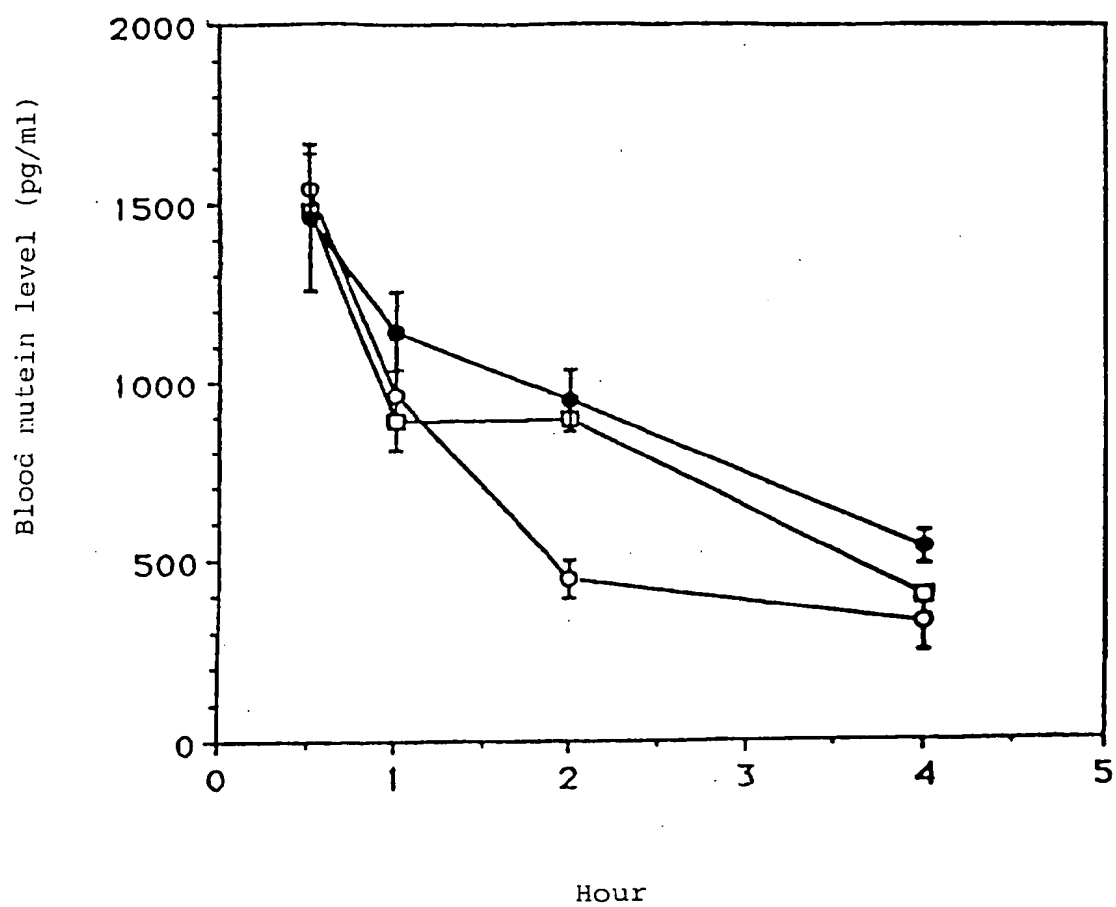


Fig. 2

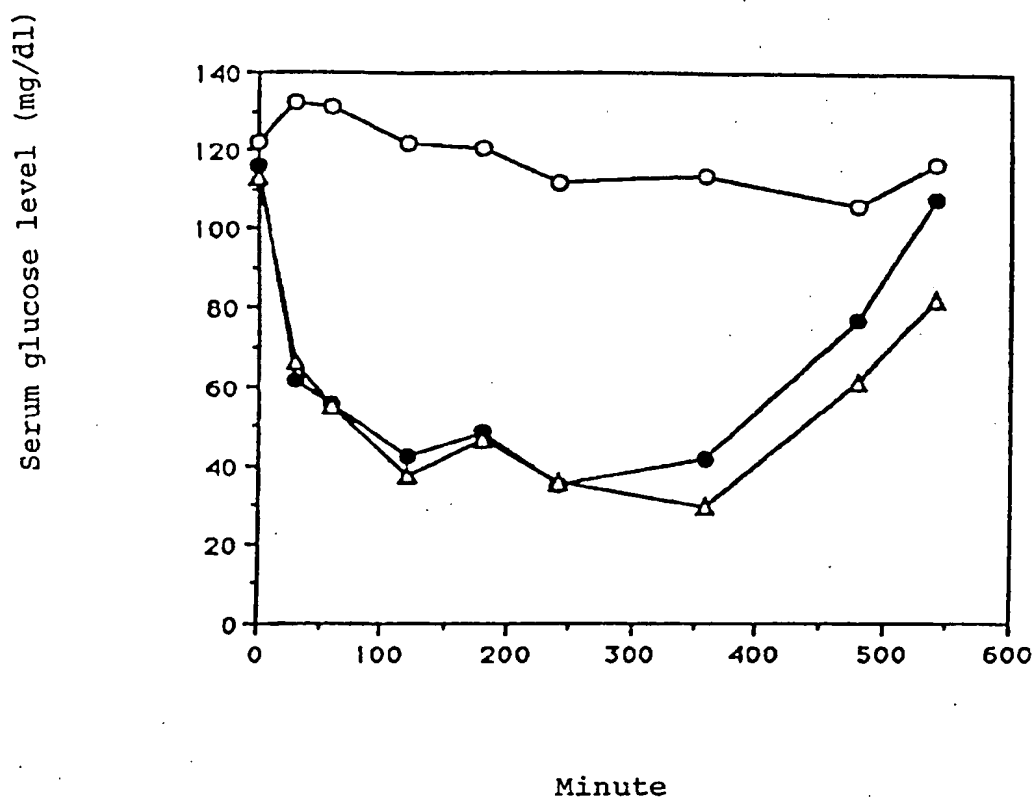


Fig. 3

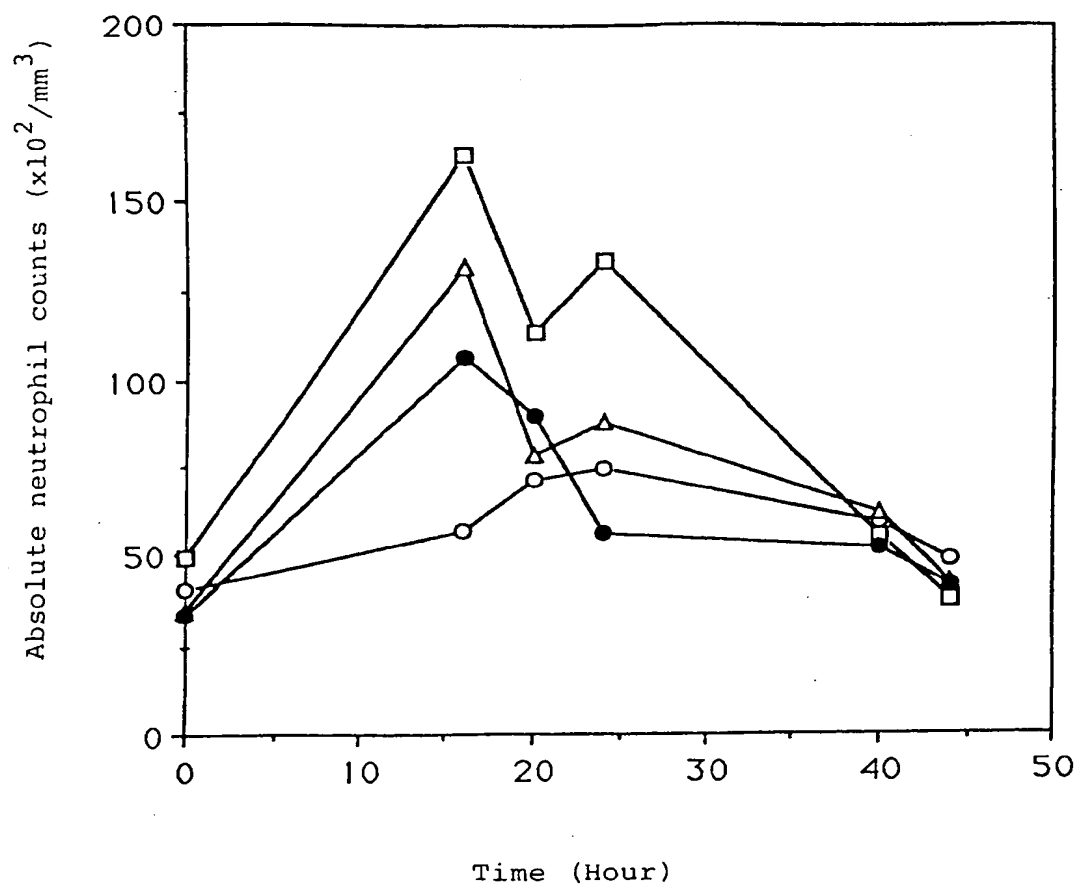


Fig. 4

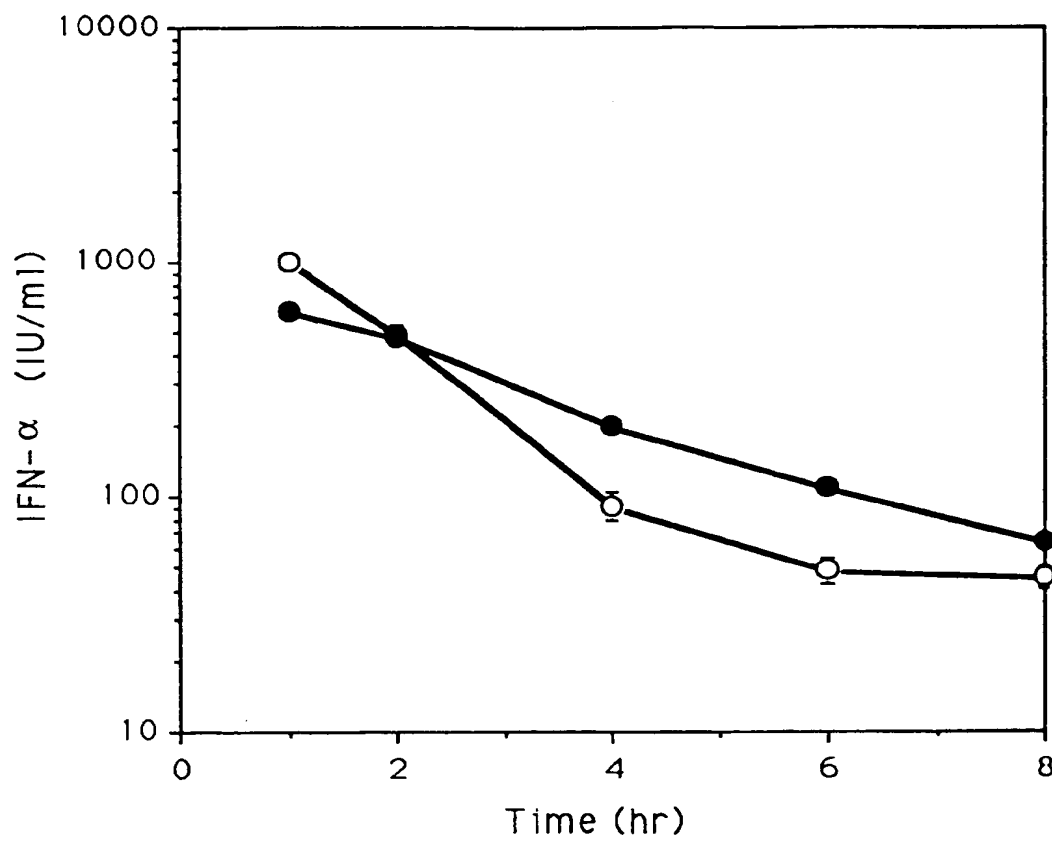
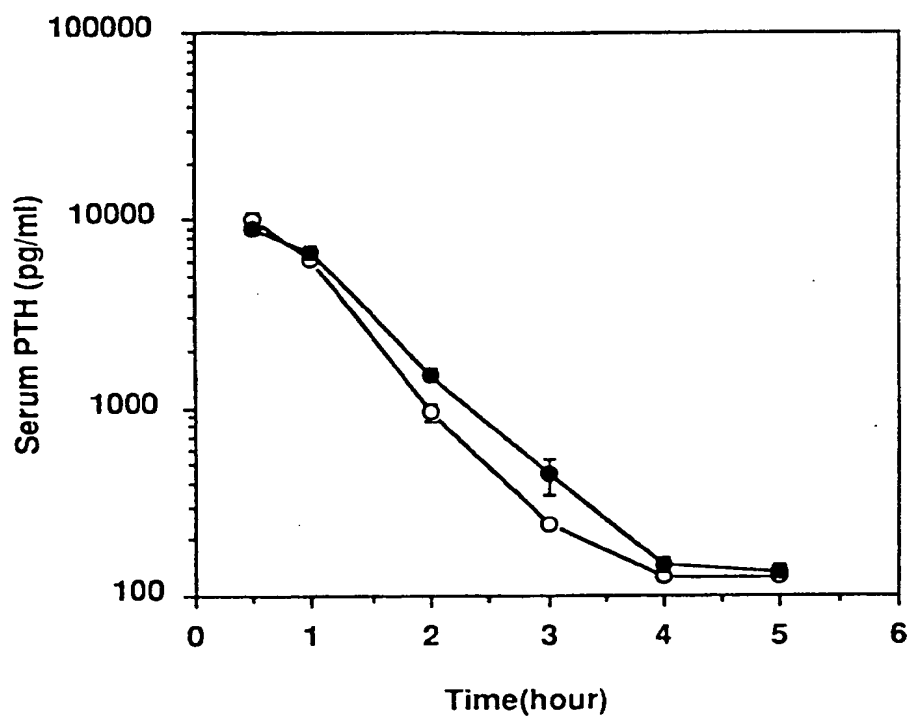


Fig. 5





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Application Number

EP 92 11 1469

DOCUMENTS CONSIDERED TO BE RELEVANT			
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The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 08-10-1992	Examiner SCARPONI U.
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X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	

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